Title: Leaf reflectance spectroscopy captures variation in carboxylation capacity across species, canopy environment, and leaf age in lowland moist tropical forests

Running Head: Remote estimates of leaf carboxylation capacity

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Key words: vegetation spectroscopy, gas exchange, plant functional traits, seasonality, Earth system models

Type of Paper: Original Research

Words/Figures Record:
Word count: summary (200 words); maintext and acknowledgements (6818 words)
Figures: 4 main figures + 3 supplementary figures
Tables: 2 supplementary tables

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Summary

- Understanding the pronounced seasonal and spatial variation in leaf carboxylation capacity ($V_{c,max}$) is critical for determining terrestrial carbon cycling in tropical forests. However, an efficient and scalable approach for predicting $V_{c,max}$ is still lacking.
- Here we tested the ability of leaf spectroscopy for rapid estimation of $V_{c,max}$. We estimated $V_{c,max}$ using traditional gas exchange methods, and measured reflectance spectra and leaf age in leaves sampled from tropical forests in Panama and Brazil. We used these data to build a model to predict $V_{c,max}$ from leaf spectra.
- Our results demonstrated that leaf spectroscopy accurately predicts $V_{c,max}$ of mature leaves in Panamanian tropical forests (R^2=0.90). However, this single-age model required recalibration when applied to broader leaf demographic classes (i.e. immature leaves). Combined use of spectroscopy models for $V_{c,max}$ and leaf age enabled construction of the $V_{c,max}$-age relationship solely from leaf spectra, which agreed with field observations. This suggests that the spectroscopy technique can capture the seasonal variability in $V_{c,max}$, assuming sufficient sampling across diverse species, leaf ages and canopy environments.
- This finding will aid development of remote sensing approaches that can be used to characterize $V_{c,max}$ in moist tropical forests and enable an efficient means to parameterize and evaluate terrestrial biosphere models.

Introduction

Projecting the fate of terrestrial ecosystems under a changing climate requires knowledge of plant physiology and ecology, and representation of that process knowledge in Earth system models (ESMs). In particular, photosynthesis is a critical process to represent accurately. In the most widely used model of photosynthesis, the rate of CO_2 assimilation is determined by the maximum carboxylation rate of the enzyme Rubisco ($V_{c,max}$), the rate of RuBP regeneration through electron transport, and in some models, the utilization of triose phosphates (Farquhar *et al.,* 1980; Sharkey *et al.,* 2007). The $V_{c,max25}$, which is $V_{c,max}$ standardized to a reference temperature of 25°C (Bernacchi *et al.,* 2013), is a key parameter at the heart of many ESMs, and variation in this parameter has repeatedly been shown to be the source of a large fraction of overall model uncertainty (e.g. Bonan *et al.,* 2011; Rogers, 2014; Rogers *et al.,* 2017a; Walker *et al.,* 2017; Ricuitto *et al.,* 2018). Accurate and comprehensive observations of the biogeography, ecology and overall distribution of $V_{c,max25}$ is thus a critical research need for improving understanding and model predictions of photosynthesis at local, regional and global scales.

Most ESMs currently represent $V_{c,max25}$ with a single static value for each plant functional type (Bonan *et al.,* 2011; Rogers, 2014). This assumption is most questionable for the tropical forest biome where forests hold enormous plant functional diversity (Condit *et al.,* 2005; Steege *et al.,* 2013; Asner *et al.,* 2014) that includes diversity in photosynthetic capacity (Norby *et al.,* 2017; Walker *et al.,* 2017). Furthermore, for a given species, $V_{c,max25}$ has been shown to vary greatly with leaf development, growth temperature, and water and nutrient availability (Medlyn *et al.,* 1999; Wilson *et al.,* 2001; Kenzo *et al.,* 2006; Kattge & Knorr, 2007; Ali *et al.,* 2015; Norby *et al.,* 2017; Albert *et al.,* 2018; Kumarathunge *et al.*, 2019; Smith *et al.*, 2019). Recently it was shown that the seasonality of photosynthesis in Amazonian evergreen forests, a ~4 Gt yr^{-1} fluctuation in CO_2 assimilation (estimated using the envelop calculation approach to extend existing site-level study in Amazon to the entire Amazon basin), is driven by the replacement of old leaves that have a low $V_{c,max25}$ with recently matured leaves that have a higher $V_{c,max25}$ (Wu *et al.,* 2016; Albert *et al.,* 2018). These studies also demonstrated that it is critical to quantify leaf age and couple this information with estimates of $V_{c,max25}$ to more accurately model leaf CO_2 assimilation by tropical forests. This result is likely also applicable to other vegetative biomes that contain plants with long-lived leaves (e.g. needle-leaf evergreen) or with significant seasonal variation (Wilson *et al.,* 2001; Han *et al.*, 2008; Muraoka *et al.,* 2010; Niinemets, 2016).

However, scaleable $V_{c,max25}$ data to enable this approach in models is lacking and tedious to collect in the tropics, which is reflected in the very poor geographical coverage of tropical plants in plant trait databases (Kattge *et al.,* 2011; Schimel *et al.,* 2015; Diaz *et al.,* 2016).

Typically, leaf-level $V_{c,max25}$ is estimated by fitting a model to a photosynthetic CO_2 response curve measured using gas exchange in a process that can take over forty-five minutes for a single measurement (Long & Bernacchi, 2003). Although faster methods of estimating $V_{c,max}$ have recently been described (DeKauwe *et al.,* 2016; Stinziano *et al.,* 2017), gas exchange measurements remain challenging in natural systems such as tropical forests where many species must be characterized at large scales. Canopy access presents an additional challenge in some systems, including tropical forests where canopy height can exceed thirty meters, requiring canopy cranes or tree climbing, which may be prohibitively time-consuming or expensive. Moreover, reliably tracking leaf age, i.e. using the leaf tagging method with intensive *in-situ* revisits and surveys (Reich *et al.,* 2004; Wu *et al.,* 2017), coupled with leaf gas exchange measurements, adds another level of difficulty. This challenge is particularly acute for moist tropical forests in which periods of new leaf production can last from a week up to a year, and different tree species have distinct and often irregular new leaf production patterns both in their timing and amplitude (Reich *et al.,* 2004; Lopes *et al.,* 2016; Xu *et al.,* 2017). Within this context, researchers require methods that allow rapid estimation of $V_{c,max25}$ and leaf age that can be applied to tall trees in natural systems, including remote tropical forests.

Recent advances in vegetation spectroscopy offer a promising solution given that this approach tightly connects leaf optical properties with their chemical composition, cell structure and physiological properties (Curran, 1989; Elvidge, 1990; Kokaly *et al.,* 2009). As such, spectroscopy has been receiving increasing attention from a broader science community, including those from plant ecophysiology, functional trait ecology, and evolution (Serbin *et al.,* 2012; Asner *et al.,* 2016; Schneider *et al.,* 2017; Schweiger *et al.,* 2018). For example, recent studies suggest that leaf $V_{c,max}$ can be estimated accurately and rapidly based on leaf reflectance spectra (e.g. Doughty *et al.,* 2011; Serbin *et al.,* 2012; Ainsworth *et al.,* 2014; Barnes *et al.,* 2017; Dechant *et al.,* 2017; Yendrek *et al.,* 2017; Silva-Perez *et al.,* 2018). In addition, two recent studies have also shown that leaf spectroscopy provides an accurate, rapid means to assess leaf age at both individual and community scales (Chavana-Bryant *et al.,* 2017; Wu *et al.,* 2017). Furthermore, some studies also suggest that it is possible that spectroscopy-based models of leaf

$V_{c,max25}$ and age could be extended to the canopy scale by leveraging imaging spectroscopy instrumentation on tower, unmanned aerial systems, and manned airborne platforms (Serbin *et al.,* 2015; De Moura *et al.,* 2017). These developments highlight the potential to map changes in $V_{c,max25}$ and leaf age over unprecedented spatial and temporal scales. However, the ability of spectra to predict the variability in $V_{c,max25}$ across these multiple axes of variation (i.e. species, canopy position, leaf age, and forest sites) has not been tested, and a spectroscopy-based approach that can account for variation in both leaf age and $V_{c,max25}$ has not been developed.

In this study we collected leaf gas exchange, reflectance spectroscopy and leaf age data from three lowland moist tropical forests. Our goal was to develop a single spectroscopic approach capable of capturing the variation in $V_{c,max25}$ among leaves of different ages from a range of species and canopy environments (i.e. variation in canopy height and sunlit and shaded environments) in lowland moist tropical forests. We asked two main questions: (1) Can the spectra-$V_{c,max25}$ relationship for mature leaves also be applied to leaves of other leaf demographic classes (e.g. immature leaves), and if not, can a new spectra-based model of $V_{c,max25}$ be developed that performs well across all leaf ages? (2) Can leaf spectra information alone enable accurate estimation of the developmental trajectories of $V_{c,max25}$, i.e. the $V_{c,max25}$–leaf age relationship? By answering these questions, we hope to understand if the spectroscopy approach can be used to capture the $V_{c,max25}$ variability in moist tropical forests, thereby accelerating current capacity to parameterize ESMs for improved projection of terrestrial carbon and water fluxes in the context of a changing climate.

Materials and Methods

Site descriptions

This study used data collected from three lowland seasonal moist tropical forests, including two crane sites in the Republic of Panama and one site in Brazil. The two sites in Panama include a seasonally dry forest in the Parque Natural Metropolitano (PNM; 8.9950° N, 79.5431° W) near Panama City and a wet evergreen forest in the San Lorenzo Protected Area (SLZ; 9.2810° N, 79.9745° W), Colon Province. Both sites are dominated by clay soil (Turner & Romero, 2009). Mean annual air temperature at both sites is 26 °C (1998–2015), and mean annual precipitation is 1826 mm yr^{-1} and 3286 mm yr^{-1} for PNM and SLZ, respectively, with a 4-month-long dry season (precipitation < 100 mm per month) from January to April each year. At

each site, the Smithsonian Tropical Research Institute maintains a canopy-access crane that enables access throughout the canopy of these forests. The site in Brazil (2.8500° S, 54.9667° W) is located around the K67 eddy covariance site in Tapajos National Forest, near Santarem, Para, Brazil. Part of the Brazilian Large Scale Biosphere-Atmosphere Experiment in Amazonia (LBA; Davidson *et al.,* 2012), this site sits on a well-drained clay-soil plateau. Multiple-year (2002–2005) mean annual air temperature is 26 °C (Hutyra *et al.*, 2007). Mean annual precipitation (1998–2013) is 2022 mm yr^{-1} with a 5-month-long dry season from mid-July to mid-December each year. Single rope access techniques were used to climb into and access individual crowns of canopy trees (Albert *et al.,* 2018). For details about forest composition and structure of the sites in Panama see Wright *et al* (2003), and of the site in Brazil see Rice *et al* (2004). For information about soil fertility of the sites in Panama see Turner & Romero (2009), and of the site in Brazil see Nepstad *et al* (2002).

Plant materials

Sixteen canopy tree species from the two sites in Panama (n=8 for SLZ and n=8 for PNM) and five canopy tree species from the Brazilian site were selected for intensive field measurements of leaf gas exchange, reflectance spectra and traits (i.e. leaf mass per area, LMA; Table S1). Sampled leaves were classified into two main age classes: immature leaves (<2 months; corresponding to the leaves from emergence up to fully-expanded, but not fully green, thickened, or physiologically matured) or mature leaves (≥2 months old), following the similar age categories as presented in Coley (1983), Wu *et al* (2016), and Albert *et al* (2018). This classification of leaf age is very similar to the three-age-category (young, mature and old) used in Wu *et al* (2016), except that we grouped mature and old age classes together into a single age class, mature. The reason of doing this is because we didn't track leaf age as frequently in Panama as that in Brazil (Wu *et al.,* 2017), and therefore lacked the resolution to differentiate three age classes. Field measurements in Panama were conducted in the 2016 and 2017 dry seasons on sunlit upper canopy foliage. In the 2016 field campaigns in mid-February and mid-April we sampled the dominant leaf class(es) from eight trees at each site. In February 2017 the measurements included both age classes if present within the top meter of a sunlit branch from four canopy tree species at the SLZ site (Table S1). Field measurements of canopy trees in Brazil, including leaves of both age classes from sunlit and shaded branches, were conducted

during the 2012 dry season field campaign from mid-August until early-December and a 2013 dry season campaign in August, using single-rope access techniques. For more details on surveyed tree species, please refer to our Table S1 & Albert *et al* (2018).

Field measurements

(1) Leaf gas exchange. We used six portable gas exchange systems in Panama and two in Brazil (LI-6400XT, Li-COR Inc., Lincoln, NE, USA). Measurements of the response of net assimilation rate (A) to intra-cellular CO_2 concentration (C_i), commonly known as A-C_i response curves, were conducted on leaves from cut branches. In Panama, all branches were sampled before dawn using the canopy cranes. We took steps to avoid inducing xylem embolism when collecting branches, and when it was possible, the initial cut was made under water by bending the branch section into a bucket filled with water. Otherwise, the initial cut was made in the air and then a second cut was made underwater approximately 1 meter from the initial cut. In all cases, several cuts were made sequentially closer to the branch tip to relax xylem tension, following the protocol as described by Sperry (2013). Samples were stored in individual buckets and kept in deep shade until used for measurements, which was normally within four hours after harvesting. Measurements of A-C_i curves closely followed Rogers *et al* (2017b), with the reference CO_2 concentration controlled as follows: 400, 325, 250, 175, 100, 65, 40, 400, 400, 400, 475, 575, 675, 800, 1000, 1400 and 1800 μmol mol^{-1}, while holding leaves at 31±2 °C and 83±5% relative humidity under saturated light condition (i.e. 2000 μmol m^{-2} s^{-1}). In Brazil, branch samples were collected via tree climbing, and gently lowered to the ground with ropes in the morning, and re-cut under water within 15 minutes. Samples were stored in individual buckets and kept in deep shade until used for measurements (typically within four hours after harvesting). Full details of leaf gas exchange measurements from Brazil are in Albert *et al* (2018). In brief, the protocol was similar as that was described above except that in Brazil, the reference CO_2 concentration was controlled as follows: 400, 100, 50, 100, 150, 250, 350, 550, 750 μmol mol^{-1}, and then increased by increments of between 200 to 500 to reach saturation at around 2000, and leaf temperature was controlled at 31±2 °C and chamber humidity was controlled at 46±11%.

Prior to curve fitting, quality control procedures for gas exchange measurements from all sites excluded values associated with instrument error and other known artifacts, such as

spurious logs and data where leaks were clearly apparent, as described in Rogers *et al* (2017b) and Albert *et al* (2018). Finally, apparent maximum carboxylation capacity standardized to a reference temperature of 25°C ($V_{c,max25}$) was estimated using the kinetic constants and temperature response functions presented by Bernacchi *et al* (2013) as described by Rogers *et al* (2017b). A total of 186 leaves with estimated $V_{c,max25}$ in Panama and 81 leaves in Brazil were used in this study, with species-specific mean and standard deviation summarized in Table S1.

(2) Leaf spectra. Following leaf gas exchange measurements, we kept the branches in water and within two hours harvested the leaf and immediately measured leaf reflectance spectra and fresh mass. Leaf reflectance at the Panamanian sites was measured using a Spectra Vista Corporation (SVC) HR-1024i (SVC, Poughkeepsie, NY, USA; spectral range: 350–2500nm; spectral resolution: 3.5 nm at 700nm, 9.5 nm at 1500 nm, and 6.5 nm at 2100 nm) together with the SVC LC-RP-Pro foreoptic. Similarly, leaf reflectance of Brazilian plants was measured using a FieldSpec® Pro spectrometer (Analytical Spectra Devices, ASD, Boulder, CO, USA; spectral range: 350–2500nm; spectral resolution: 3 nm at 700nm, 10 nm at 1400 nm, and 10 nm at 2100 nm) together with a ASD leaf clip attached to a plant probe assembly. In both cases, the reflectance probes contained internal, calibrated light sources to illuminate the samples during spectral collection. The leaf probe was used together with a black background for leaf reflectance measurements. To avoid the excessive heat loads while ensuring the reliable spectral collection we set the ASD integration time to 100 milliseconds per scan and each collected spectra was an average of 10 scans, while with the SVC we used a 1 second collection time and used the spectrometer's automatic integration optimization. This approach matches that of Serbin *et al* (2012), which originally highlighted the concerns of the excessive heat loads on the data quality of leaf spectra collected. For each leaf, reflectance spectra were measured on 1–6 different parts of the leaf adaxial surface depending on leaf size, and then averaged to determine the mean optical properties across all wavelengths.

(3) Leaf traits. Leaf mass per area (LMA; g m^{-2}) was also measured to assess the diversity of plant species that we sampled in terms of the LMA trait space. In Panama, we sampled a known leaf area using cork borers. The samples were dried to constant mass at 70°C. We then determined dry mass with a precision balance (Fisher Science Education, Model SLF303, Hanover Park, IL, USA) to calculate LMA. In Brazil, LMA was derived from area (using a

Canon LiDE 120 scanner) and dry weight (also using a precision balance from Fisher Science Education, Model SLF303, Hanover Park, IL, USA) oven-dried at 60°C for over 72 hours.

(4) Leaf age. In Brazil, in field campaigns conducted in August–September 2013, November 2013, March 2014 and July–August 2014, we selected seven trees of different species (see Table 1 in Wu *et al.,* 2017) for precise *in-situ* leaf age monitoring. Leaf age monitoring was carried out by using metal tags and *in-situ* photo documentation (e.g. Fig. S1 in Wu *et al.,* 2017). Monitoring began in August–September 2013, when most sampled trees were flushing new leaves, and was continued periodically throughout the annual cycle. Through this age-tagging technique, we accurately tracked leaf age from leaf emergence at budburst (0 day) up to ~400 days old. From those leaves with accurate leaf age monitoring, we then sampled a total of 759 leaves covering the entire annual cycle, and measured leaf reflectance spectra using the same ASD FieldSpec® Pro spectrometer as described above. These leaves were then used for the development of the community-level spectra-age model (Wu *et al*., 2017) and briefly summarized below. Among these seven trees surveyed for both leaf age monitoring and leaf reflectance measurements, four were the same canopy trees (including leaf samples from both sunlit and shaded microenvironments) from which we made gas exchange measurements as described above (also see Table S1).

In addition to the above-mentioned accurate leaf age monitoring, we also took RGB photos for all leaves used for leaf reflectance measurements in both Panama and Brazil. These RGB photos together with other related information, e.g. visual assessment of color, size and rigidity of the leaves, and relative positions and bud scars (when present) within a 1-meter branch length, were then used to classify these leaves into two different age categories: immature and mature leaves.

(5) Spectra-$V_{c,max25}$ analysis. For all field-based spectral and gas exchange measurements in Panama and Brazil, only the plant species with both leaf reflectance spectra and $V_{c,max25}$ were selected (which excluded 25 measurements in Panama and 12 measurements in Brazil with only leaf gas exchange). Finally, we performed a filtering of outliers of combined spectra-$V_{c,max25}$ datasets (which removed ~5% of data). The outlier detection method implemented here was originally used in Wu *et al* (2017), which adapted an outlier detection module from "libPLS" (accessed at http://www.libpls.net/), using the Monte-Carlo sampling method (Xu & Liang, 2001) for automatic outlier detection. After the data filtering, the Panamanian dataset had n=151

measurements, including 110 mature leaves from all 16 species and 41 immature leaves from 9 of the 16 species, which accounts for 94% of all measurements with both spectral and leaf gas exchange in Panama. Brazilian dataset had *n*=65 measurements, including 44 mature leaves and 21 immature leaves from all 5 species, which accounts for 94% of all measurements with both spectral and leaf gas exchange in Brazil.

All the data sources associated with this study including gas exchange data, leaf spectral data, leaf trait, and leaf age information were summarized in Table S2.

Partial least-squares regression (PLSR) modeling of spectra-$V_{c,max25}$ and spectra-age

To relate the variability in $V_{c,max25}$ across tree species, leaf age, canopy position, and forest sites with the variability in leaf reflectance spectra and to infer leaf age from leaf reflectance spectra, we utilized a PLSR modeling approach (Geladi & Kowalski, 1986; Wolter *et al.,* 2008) using the "plsregress" function in Matlab (Mathworks, Natick, MA, USA) as described in De Jong (1993) and Rosipal & Kramer (2006). PLSR is a commonly-used approach in spectroscopy and chemometric analyses given its ability to handle high predictor collinearity and a large number of predictor variables that may exceed the number of observations. PLSR accounts for these challenges by reducing the number of predictor variables down to relatively few, orthogonal latent variables, each composed of a weighted sum of the original variables (Geladi & Kowalski, 1986; Wolter *et al.,* 2008). Moreover, PLSR accounts for measurement error in the predictor variables (i.e. leaf hyperspectral reflectance).

Our PLSR model development has been described previously (Wu *et al.,* 2017) and is briefly summarized here. We first applied a square root transformation to the $V_{c,max25}$ data and leaf age data to reduce the right skewness distribution of the original data (e.g. Figs. S1a,b and S2a,b) and satisfy the normal distribution assumption of PLSR analysis. We then performed a one-time, random, stratified separation of the full dataset into calibration (two thirds) and independent validation (one third) subsets; stratification insured that each subset included leaf samples of each age category, of each species, and (when appropriate) of each canopy position. Next, we randomly selected 70% of the calibration data subset, and fit the PLSR model of spectra-$V_{c,max25}$, with this random selection, repeating this 100 times and for each permutation applying the model to predict the corresponding independent 30% of the calibration data. To avoid the potential to over-fit the spectra-based calibration model, we optimized the number of

PLSR latent variables by choosing the number of latent variables that minimized the root mean square error (RMSE) from predicting the remaining 30% of the calibration data over the 100 permutations (Chen *et al.,* 2004; e.g. Fig. S1c). Meanwhile, we also determined the mean and standard deviation of the distribution of PLSR coefficients generated by the 100 PLSR fits corresponding to the optimal number of latent variables, which were used in the final spectra-$V_{c,max25}$ model (e.g. Fig. S1d).

Finally, we quantified the performance of this spectra-$V_{c,max25}$ model using the independent validation dataset. We used three main evaluation metrics: the coefficients of determination (R^2), RMSE, and the regression bias. All model results presented in this study are shown in the original $V_{c,max25}$ units rather than the square root transformed unit that is the initial output of the PLSR model. The same spectral analytical approach was applied to the Brazilian spectra-age dataset to derive the community-level spectra-age model (Fig. S2 and Wu *et al*., 2017). All of the code used for model development and data analysis were developed in Matlab (Mathworks, Natick, MA, USA).

Generalizability of spectra-$V_{c,max25}$ relationship

We explored whether the spectra-$V_{c,max25}$ relationship can be generalized across species, leaf age and canopy environment through two tests. In the first test, we developed a spectra-based PLSR model using two-thirds of the Panamanian data for mature leaves to train the model (including all 16 species). We then applied this model to the remaining Panamanian dataset of mature leaves, as well as to the independent validation dataset of Panamanian immature leaves, and Brazilian mature and immature leaves. Through this test, it would enable us to assess whether the spectra-$V_{c,max25}$ relationship of mature leaves can also be applied to leaves of immature age class or leaves of a different forest site in Brazil. In the second test, we developed a new spectra-based model in which all our datasets (including two-thirds of all leaves from both Panama and Brazil) were used to train the model. Model performance was evaluated using the remaining, independent datasets, which are the same as that were used in the first test. Through this test, it would enable us to assess whether a single spectra-$V_{c,max25}$ relationship can be applied to leaves of both leaf age classes and different forest sites.

Spectral-based seasonal variability in $V_{c,max25}$, or $V_{c,max25}$-age relationship, using a combination of spectral models of $V_{c,max25}$ and leaf ae

With the developed spectra-$V_{c,max25}$ model and spectra-age model as described above, we then used these two models in combination to explore whether leaf spectra information alone can be used to reconstruct the life history variability in leaf $V_{c,max25}$ for tropical trees. The spectra-age dataset in Brazil were used for this test, as the dataset covered the leaf spectra throughout their life cycles (Wu *et al.*, 2017) while having some ground truth of $V_{c,max25}$ derived from gas exchange measurements (Albert *et al.*, 2018). These two models are both at the community level and their model regression coefficients are respectively shown in Figs. S1d and S2d. The models were driven by the input of leaf spectral reflectance only but can predict leaf $V_{c,max25}$ and leaf age respectively (see the provided sample Matlab script). By combining the model output of $V_{c,max25}$ and age together, the spectroscopy approach was thus used to estimate the $V_{c,max25}$-age relationship, or the life history variability in leaf $V_{c,max25}$ with leaf age.

Results

As shown in Fig. 1 and Table S1, we found large variability in leaf $V_{c,max25}$ for the surveyed 21 tropical trees from three tropical forest sites: field-measured $V_{c,max25}$ ranged from 7-102 μmol CO_2 m^{-2} s^{-1}. These surveyed trees also spanned a very large variation in leaf morphology, as shown in the observed LMA trait space (i.e. 70-213 g m^{-2}). We also found that the variation in $V_{c,max25}$ is attributable to species (Fig. 1a and Table S1), leaf age (mature vs. immature; Fig. 1a), canopy environment (sunlit vs. shaded; Fig. 1a), and also forest sites (Fig. 1b). The large intra-specific variation in $V_{c,max25}$ is primarily associated with leaf age, and the large inter-specific variation in $V_{c,max25}$ is attributable to both species difference but also the forest sites. Such large variation in $V_{c,max25}$, especially the variation with leaf age, will make the traditional approaches for measuring this diversity challenging due to the requirement for lots of measurements.

We examined our first question of whether the spectroscopy approach can be an efficient, alternative means to help estimate $V_{c,max25}$ across species, leaf age, canopy environment and forest sites through the two tests (see Methods above). In the first test, we found that the model based solely on Panamanian mature leaves was able to predict the field-observed $V_{c,max25}$ of independent Panamanian mature leaves with very high accuracy (R^2=0.90; RMSE=5.9 μmol

CO_2 m^{-2} s^{-1}; *n*=36 leaves; Fig. 2a), suggesting a tight covariation in the spectra-$V_{c,max25}$ relationship for Panamanian forests across a diverse range of tree species, canopy heights and leaf traits (Table S1). However, this model did not perform as well when applied to a dataset of independent, Panamanian immature leaves from a subset of the same trees (*n*=9 species; Table S1), with the model fit having a marked deviation from the 1:1 line (between modeled and observed $V_{c,max25}$) and displaying poor predictive ability (R^2=0.02; RMSE=20.7 μmol CO_2 m^{-2} s^{-1}; *n*=14 leaves; Fig. 2b). When the model of Panamanian mature leaves was applied to independent, Brazilian mature and immature leaves the model performance was also poor (R^2=0.23; RMSE=38.8 μmol CO_2 m^{-2} s^{-1}; *n*=22 leaves; Fig. 2c). This showed that the model developed from Panamanian mature leaves could neither be directly applied to immature leaves of the same trees nor the leaves sampled from other tropical forests without marked reduction in predictive power (Fig. 2d).

In the second test, we found that the new model trained on the data from all species, leaf ages, canopy environment, and forest sites performed dramatically better across the whole range of leaf types—immature leaves in Panama (R^2=0.89; RMSE=3.9 μmol CO_2 m^{-2} s^{-1}; *n*=14 leaves; Figs. 3 and S3a) and all leaf ages from Brazil (R^2=0.68; RMSE=5.9 μmol CO_2 m^{-2} s^{-1}; *n*=22 leaves; Figs. 3 and S3b)—at the cost of only slightly lower prediction of mature Panamanian leaves (R^2=0.86; RMSE=7.7 μmol CO_2 m^{-2} s^{-1}; *n*=36 leaves; Figs. 3 and S3c). In addition, compared with our initial model of Panamanian mature leaves (Fig. 2, first test), the new model (Fig. 3, second test) also significantly reduced the uncertainty in model predicted $V_{c,max25}$, as indicated by the horizontal error bars shown in Figs. 2 and 3. This analysis demonstrated that with sufficient leaf samples to train the spectra-$V_{c,max25}$ relationship over the full trait space, a general spectra-based $V_{c,max25}$ model can be derived across species, leaf age, canopy environment, and forest sites.

We next examined our second question: whether the spectroscopy approach alone is sufficient to simulate the life history trajectories of $V_{c,max25}$, or the $V_{c,max25}$-age relationship. To do this, we applied two models (i.e. the community-level spectra-$V_{c,max25}$ model and spectra-age model) to the spectra-age dataset in Brazil (see Methods). The results showed that there was large variability in leaf $V_{c,max25}$ across leaf life cycles (i.e. from 15-days old to 400-days old), but that the spectroscopy approach presented here was able to track leaf age dependent variation in $V_{c,max25}$ (Fig. 4), particularly during the period from emergence to physiological full-maturity

(from 15 to 150 days). With continued aging and senescence (>150 days), there was larger deviation between spectra-predicted and field-observed $V_{c,max25}$. This might largely be because our datasets had poor coverage of older leaves (from 150 days to 400 days), and thus the model for the senescent leaves is not well calibrated.

Discussion

Leaf carboxylation capacity $V_{c,max25}$ is central to the estimation of photosynthetic CO_2 uptake by tropical forests in ESMs. In this study, we demonstrated that the spectroscopy approach is able to accurately predict $V_{c,max25}$ across species with a large range in LMA trait space, leaf age, canopy position and height, and forest sites (Fig. 3 and Table S1). We also showed that the combined application of spectroscopy models of leaf $V_{c,max25}$ and age are sufficient to track life time variation in leaf $V_{c,max25}$ (Fig. 4). This represents a significant breakthrough in our ability to rapidly estimate and potentially map $V_{c,max25}$ in high spatial and temporal resolution in tropical forests.

Consistent with previous studies (Field, 1983; Sobrado, 1994; Wilson *et al.,* 2001; Kitajima *et al.,* 2002; Kenzo *et al.,* 2006; Pantin *et al.,* 2012; Albert *et al.,* 2018), we found large variability in leaf $V_{c,max25}$ (i.e. 7-102 μmol CO_2 m^{-2} s^{-1}) with species, leaf age and canopy environment across 21 species sampled from the three lowland moist tropical forests. Such a wide range of variability in $V_{c,max25}$ is comparable with previous studies at the same forest sites with larger sample size and a focus on mature leaves (i.e. 15-75 μmol CO_2 m^{-2} s^{-1} from 65 species in Panama, Norby *et al.*, 2017; 10-80 μmol CO_2 m^{-2} s^{-1} from 38 species in Brazil, Domingues *et al.*, 2014). It is also comparable with the observations from other moist tropical forest sites in Brazil (Carswell *et al.*, 2000), Peru (Bahar *et al.*, 2017) and Africa (Domingues *et al.*, 2010).

These past studies together with our findings also suggest that leaf age is one of the most important sources of variation in $V_{c,max25}$, which is clearly shown in Figs. 1 and 4. Since $V_{c,max25}$ can change markedly with leaf age and our observed $V_{c,max25}$ variability spanned almost the same range (13-90 μmol CO_2 m^{-2} s^{-1}) as that used to represent global variation in $V_{c,max25}$ in current ESMs (Rogers, 2014), it further suggests the importance of including such age-dependent $V_{c,max25}$ variation in future model formulations. The high $V_{c,max25}$ variability associated with leaf age also highlights the value of our developed spectroscopy approach to enable rapid estimations of

$V_{c,max25}$: the spectroscopy approach only takes a few seconds to estimate $V_{c,max25}$, once the model has been recalibrated, while the conventional approach using leaf gas exchange measurement of a photosynthetic CO_2 response curves takes about an hour (or more). Furthermore, the conventional approach cannot simultaneously derive leaf age—an important piece of information needed to improve model representation of photosynthesis, especially in species-rich, evergreen tropical forests (Kim *et al.,* 2012; Wu *et al.,* 2016).

Here we have demonstrated that leaf spectroscopy offers a tool to rapidly capture multiple important axes of variation in $V_{c,max25}$: A single spectra-based model of leaf $V_{c,max25}$ was able to predict leaf $V_{c,max25}$ across tropical tree species with a very large variation in LMA trait space, leaf age, canopy position and forest sites with high confidence (Fig. 3 and Table S1). This finding validates pioneering work that demonstrated not only the feasibility of using leaf spectra to model $V_{c,max}$ under a narrow range of species and conditions (Doughty *et al.,* 2011; Serbin *et al.,* 2012), but also dramatically expands our confidence to use the improved spectra-$V_{c,max25}$ model across a wide range of species, leaf developmental stages and locations.

So what is the underlying mechanism for such a tight covariation between leaf spectra and $V_{c,max25}$ across the various axes of variation (i.e. species, leaf age, canopy positions and forest sites) we considered in this study? There are at least two potential hypotheses.

The first is that the tight coordinated variation in leaf $V_{c,max25}$ and spectra was entirely based on their relationships with leaf nitrogen content (e.g. Dechant *et al.*, 2017). The theory underlying this hypothesis is that leaf $V_{c,max25}$ is tightly coupled with the nitrogen content in Rubisco which comprises the largest fraction of N invested in a single enzyme within a leaf (e.g. Jacob *et al.*, 1995; Onoda *et al.*, 2004; Dong *et al.*, 2017; Scafaro *et al.*, 2017; Evans & Clark, 2019). This hypothesis seems moderately supported by two previous studies conducted at our study sites (that focused on mature leaves with larger sample size), including significant, but only modest correlations of $V_{c,max25}$ with leaf nitrogen (R^2=0.31; Norby *et al.*, 2017; R^2=0.33; Dominguez *et al.*, 2014). Given that leaf spectra can efficiently capture variation in leaf nitrogen content (e.g. Asner & Martin, 2008; Serbin *et al.*, 2014; Dechant *et al.*, 2017), it is expected that leaf spectra can also be used to predict leaf $V_{c,max25}$. However, this hypothesis does not stand up to further scrutiny for several reasons. First, if the correlation with leaf nitrogen content was the primary driver of our ability to estimate $V_{c,max25}$ with spectra, it does not explain why leaf spectra shows far higher predictive power than using leaf nitrogen content alone (R^2=0.89 for leaf

spectra in this study vs. R^2=0.31-0.33 for leaf nitrogen content as shown in Dominguez *et al.*, 2014 and Norby *et al.*, 2017). Furthermore, the $V_{c,max25}$-leaf nitrogen relationship does not always hold up at the site level level (Bahar *et al*. 2017; Rogers *et al.*, 2017b; Evans & Clark, 2019) and recent global synthesis have shown that variation in leaf nitrogen can only explain a small portion of variation in $V_{c,max}$ (Ali *et al.,* 2015; Smith *et al.,* 2019). Secondarily, many other studies suggest that in addition to leaf nitrogen content, other leaf traits, e.g. leaf phosphorus content (e.g. Walker *et al.*, 2014; Norby *et al.*, 2017), leaf chlorophyll content (e.g. Croft *et al.,* 2017), LMA (e.g. Walker *et al.*, 2014), and age (e.g. Albert *et al.*, 2018), are also related with $V_{c,max25}$, and inclusion of more traits as predictive variables can significantly improve the power of trait based model to predict $V_{c,max25}$, compared with the just one trait, leaf nitrogen content (e.g. Walker *et al.*, 2014). Finally, Serbin *et al* (2012) showed that in poplar the power of leaf N content and LMA to predict $V_{c,max}$ varied with temperature treatments, but the reflectance spectroscopy approach collapsed this variation into a single model. This suggests that the ability of the spectroscopy approach to predict $V_{c,max}$ is not entirely dependent on the ability of spectroscopy to predict leaf N content and LMA and that other factors are likely contributing to the success of the approach.

Since the correlation with leaf nitrogen might not be the only reason for the derived spectra-$V_{c,max25}$ model, this further leads to our second hypothesis: leaf $V_{c,max25}$ is correlated with multiple leaf traits and processes that determine Rubisco content and activity (e.g. leaf nitrogen content, leaf phosphorus content, leaf chlorophyll concentrations, LMA, leaf age, and many others we do not yet understand), and leaf spectra emerge from the ensemble of properties that define leaf chemical, morphological, and phenological status (e.g. Asner & Martin, 2008; Serbin *et al.*, 2014; Chavana-Bryant *et al.*, 2017, 2019). As such, leaf spectra can be used to help infer leaf $V_{c,max25}$, and are indeed a better predictor of leaf $V_{c,max25}$ (e.g. Serbin *et al.*, 2012) than alternative trait approaches that leverage well established links between $V_{c,max25}$ and just one or a few individual leaf traits (e.g. Walker *et al.*, 2014). We believe this second hypothesis offers a more plausible explanation for the power of the spectra-$V_{c,max25}$ approach. However, a more comprehensive study to elucidate the underlying mechanisms that enable the spectra-$V_{c,max25}$ model is still needed.

Our finding that the spectra-$V_{c,max25}$ model of mature leaves in Panama creates model bias when applied to Panamanian immature leaves and all Brazilian leaves is also interesting. This

observation could be attributable to different ranges in $V_{c,max25}$ for model development and validation (e.g. 17-102 μmol CO_2 m^{-2} s^{-1} for the Panamanian mature leaf model; 21-63 μmol CO_2 m^{-2} s^{-1} for Panamanian immature leaves; 7-46 μmol CO_2 m^{-2} s^{-1} for all leaves in Brazil). However, a more likely explanation is that the spectra-trait-$V_{c,max25}$ linkages (e.g. regression coefficients) vary with leaf age (e.g. Field, 1983; Wilson *et al.*, 2001; Chavana-Bryant *et al.*, 2017, 2019; Wu *et al.*, 2017) and forest sites of different soil types and fertility (e.g. Walker *et al.*, 2014; Norby *et al.*, 2017). Regardless of these potential reasons, our finding (Fig. 3) suggests that including as many axes of variation as possible in the training dataset is critical to develop a broadly applicable spectra-$V_{c,max25}$ model. Both leaf spectra and $V_{c,max25}$ can vary with vertical canopy profiles (e.g. various canopy positions including upper canopy, mid-canopy and understory trees; sunlit and shaded environments), different tropical forests types (e.g. flooded, Caatinga, second growth, and upland forests) and other leaf habits (e.g. deciduous trees) that are currently either under or not sampled in this study. Therefore we recognize that further in-depth pan-tropical and global sampling and analysis are still needed to develop a highly robust spectra-$V_{c,max25}$ model that can be applied with confidence throughout the tropics and ultimately globally. It is also worth noting that when extending the spectra-$V_{c,max25}$ model to entire vertical canopy profiles, the epiphyll effect is another issue that is needed to be considered, as many old leaves in the shaded canopy environment develop epiphylls (Sonnleitner *et al.*, 2009), which strongly impacts leaf spectral reflectance (see Roberts *et al.*, 1998).

We also showed that spectroscopy is able to simulate the life history variability in $V_{c,max25}$ with leaf age within and across tropical tree species (Fig. 4). The spectroscopy derived age-dependent $V_{c,max25}$ is also comparable with direct measurements by Wu *et al* (2016) in terms of both amplitude and the relative trend across three leaf developmental stages: young (1-2 months), mature (3-5 months) and old (6-14 months). This suggests that the spectroscopy approach can be used to track lifetime trajectories in leaf traits (including but not limited to $V_{c,max25}$ shown here), and is a marked extension of previous studies that demonstrated the feasibility of linking leaf spectroscopy to model leaf $V_{c,max25}$ (e.g. Serbin *et al.,* 2012) or leaf age (e.g. Chavana-Bryant *et al.,* 2017). Moreover, the success of spectroscopy-based $V_{c,max25}$–age relationships also highlights that the spectroscopy approach can not only be a novel means to capture the variability of $V_{c,max25}$, particularly associated with leaf age, but also rapidly generate datasets that enable the exploration of temporal and spatial variability in $V_{c,max25}$ within and

517 across species, an important piece of process knowledge that greatly needs to be incorporated in
518 future ESMs (e.g. Xu *et al.,* 2017).

519 Finally, our finding that there exists a tight relationship among leaf-level spectra, $V_{c,max25}$
520 and leaf age can likely be extended to canopy and ecosystem scales. As shown by many previous
521 theoretical and empirical studies (Asner, 1998; Asner & Martin, 2008; Ollinger, 2011; Singh *et*
522 *al.,* 2015), the fundamental changes in leaf optical properties with underlying variation in leaf
523 traits is not scale-dependent; that is leaf-level or canopy-scale spectra changes in concert with
524 leaf traits. Meanwhile, Serbin *et al* (2015) demonstrated that the imaging spectroscopy technique
525 could be effectively used to infer leaf $V_{c,max25}$ from canopy-scale hyperspectral reflectance for
526 managed agricultural sites. Given our predictive success at the leaf level, it is possible that our
527 findings could help enable predictions at the canopy level in tropical forest ecosystems.
528 Additionally, Wu *et al* (2018) connected leaf scale optical properties (i.e. reflectance and
529 transmittance) with canopy radiative transfer models to simulate the leaf age effect on canopy
530 reflectance in tropical forest ecosystems. The simulated canopy reflectance from this analysis
531 showed a good agreement with observations from high resolution WorldView-2 imagery, further
532 suggesting a potential way to scale up leaf-scale spectra-$V_{c,max25}$ relationship explored here to the
533 canopy scale. Collectively, these recent studies, together with our current findings (Figs. 3 and
534 4), help build the foundation towards the possibility of monitoring $V_{c,max25}$-age relationships at
535 canopy and ecosystem scales using state-of-the-art remote sensing technology: e.g. leveraging
536 imaging spectroscopy from unmanned aerial systems (UASs; Adão *et al.,* 2017), aircraft (e.g.
537 AVIRIS, Serbin *et al.,* 2015), and the suite of current and planned space-borne platforms (e.g.
538 EnMAP, Guanter *et al.*, 2015; HISUI, Stavros *et al.,* 2017; Surface Biology and Geology
539 mission, National Academies of Sciences E, Medicine, 2018). Imaging spectroscopy, if
540 successful in capturing $V_{c,max25}$–age relationships at canopy scales, will greatly advance our
541 capability to monitor and mechanistically understand $V_{c,max25}$ variability across both space and
542 time, providing critically important datasets to parameterize and evaluate ESMs.
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546 **Acknowledgements**

This work was supported by the Next-Generation Ecosystem Experiments–Tropics project supported by the U.S. DOE, Office of Science, Office of Biological and Environmental Research and through contract #DE-SC0012704 to Brookhaven National Laboratory. L.P.A. was supported by Voss postdoctoral research funding at Brown University. The field work in Brazil was supported by US National Science Foundation (NSF, OISE-0730305). We also acknowledge Mick Eltringham for canopy access assistance in Brazil.

Author Contributions

JW, AR, LPA, SRS and SPS designed the study. KE, SPS, AR, BTW and JW collected the field data in Panama. LPA, NP, RCO and JW collected the field data in Brazil. JW performed the data quality control and analysis. JW drafted the manuscript, and all authors contributed to the final version.

Competing Interests

The authors declare no competing interests.

References

Adão T, Hruška J, Pádua L, Bessa J, Peres E, Morais R, Sousa JJ. 2017. Hyperspectral imaging: A review on UAV-based sensors, data processing and applications for agriculture and forestry. *Remote Sensing* **9**, https://doi.org/10.3390/rs9111110.

Albert LP, Wu J, Prohaska N, Camargo PB, Huxman TE, Tribuzy ES, Ivanov VY, Oliveira RS, Garcia S, Smith MN *et al.* 2018. Age-dependent leaf physiology and consequences for crown-scale carbon uptake during the dry season in an Amazon evergreen forest. *New Phytologist* **219:** 870-884.

Ali AA, Xu C, Rogers A, McDowell NG, Medlyn BE, Fisher RA, Wullschleger SD, Reich PB, Vrugt JA, Bauerle WL *et al.* 2015. Global-scale environmental control of plant photosynthetic capacity. *Ecological Applications* **25:** 2349-2365.

Ainsworth EA, Serbin SP, Skoneczka JA, Townsend PA. 2014. Using leaf optical properties to detect ozone effects on foliar biochemistry. *Photosynthesis Research* **119:** 65-76.

Asner GP. 1998. Biophysical and biochemical sources of variability in canopy reflectance. *Remote Sensing of Environment* **64:** 234-253.

Asner GP, Martin RE. 2008. Spectral and chemical analysis of tropical forests: Scaling from leaf to canopy levels. *Remote Sensing of Environment* **112:** 3958-3970.

Asner GP, Martin RE, Tupayachi R, Anderson CB, Sinca F, Carranza-Jiménez L, Martinez P. 2014. Amazonian functional diversity from forest canopy chemical assembly. *Proceedings of the National Academy of Sciences* **111:** 5604-5609.

Asner GP, Brodrick PG, Anderson CB, Vaughn N, Knapp DE, Martin RE. 2016. Progressive forest canopy water loss during the 2012–2015 California drought. *Proceedings of the National Academy of Sciences* **113:** E249-E255.

Bahar NH, Ishida FY, Weerasinghe LK, Guerrieri R, O'Sullivan OS, Bloomfield KJ, Asner GP, Martin RE, Lloyd J, Malhi Y *et al.* 2017. Leaf-level photosynthetic capacity in lowland Amazonian and high-elevation Andean tropical moist forests of Peru. *New Phytologist* **214:** 1002-1018.

Barnes ML, Breshears DD, Law DJ, van Leeuwen WJ, Monson RK, Fojtik AC, Barron-Gafford GA, Moore DJ. 2017. Beyond greenness: Detecting temporal changes in photosynthetic capacity with hyperspectral reflectance data. *PLoS ONE* **12**, https://doi.org/10.1371/journal.pone. 0189539.

Bernacchi CJ, Bagley JE, Serbin SP, RUIZ-VERA UM, Rosenthal DM, Van Loocke A. 2013. Modelling C3 photosynthesis from the chloroplast to the ecosystem. *Plant, Cell & Environment* **36:** 1641-1657.

Bonan GB, Lawrence PJ, Oleson KW, Levis S, Jung M, Reichstein M, Lawrence DM, Swenson SC. 2011. Improving canopy processes in the Community Land Model version 4 (CLM4) using global flux fields empirically inferred from FLUXNET data. *Journal of Geophysical Research: Biogeosciences* **116**, https://doi.org/10.1029/2010JG001593.

Carswell FE, Meir P, Wandelli EV, Bonates LCM, Kruijt B, Barbosa EM, Nobre AD, Grace J, Jarvis PG. 2000. Photosynthetic capacity in a central Amazonian rain forest. *Tree Physiology* **20:** 179-186.

Chavana-Bryant C, Malhi Y, Wu J, Asner GP, Anastasiou A, Enquist BJ, Caravasi C, Eric G, Doughty CE, Saleska SR *et al*. 2017. Leaf aging of Amazonian canopy trees as revealed by spectral and physiochemical measurements. *New Phytologist* **214:** 1049-1063.

Chavana-Bryant C, Malhi Y, Anastasiou A, Enquist BJ, Cosio EG, Keenan TF, Gerard FF. 2019. Leaf age effects on the spectral predictability of leaf traits in Amazonian canopy trees. *Science of the Total Environment* **666:** 1301-1315.

Chen S, Hong X, Harris CJ, Sharkey PM. 2004. Spare modeling using orthogonal forest regression with PRESS statistic and regularization. *IEEE Transaction on Systems, Man and Cybernetics* **34:** 898-911.

Coley PD. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs* **53:** 209-234.

Condit RS, Ashton MS, Balslev H, Brokaw NVL, Bunyavejchewin S, Chuyong GB, Co L, Dattaraja HS, Davies SJ, Esufali S *et al*. 2005. Tropical tree a-diversity: results from a worldwide network of large plots. *Biologike Skrifer* **55:** 565-582.

Croft H, Chen JM, Luo X, Bartlett P, Chen B, Staebler RM. 2017. Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. *Global Change Biology* **23:** 3513-3524.

Curran PJ. 1989. Remote sensing of foliar chemistry. *Remote Sensing of Environment* **30:** 271-278.

Davidson EA, de Araújo AC, Artaxo P, Balch JK, Brown IF, Bustamante MM, Coe MT, DeFries RS, Keller M, Longo M *et al.* 2012. The Amazon basin in transition. *Nature* **481:** 321-328.

Dechant B, Cuntz M, Vohland M, Schulz E, Doktor D. 2017. Estimation of photosynthesis traits from leaf reflectance spectra: correlation to nitrogen content as the dominant mechanism. *Remote Sensing of Environment* **196:** 279-292.

De Jong S. 1993. SIMPLS: an alternative approach to partial least squares regression. *Chemometrics and Intelligent Laboratory Systems* **18:** 251-263.

De Kauwe MG, Lin YS, Wright IJ, Medlyn BE, Crous KY, Ellsworth DS, Maire V, Prentice IC, Atkin OK, Rogers A *et al.* 2016. A test of the 'one-point method' for estimating maximum carboxylation capacity from field-measured, light-saturated photosynthesis. *New Phytologist* **210:** 1130-1144.

De Moura YM, Galvão LS, Hilker T, Wu J, Saleska S, do Amaral CH, Nelson BW, Lopes AP, Wiedeman KK, Prohaska N *et al.* 2017. Spectral analysis of amazon canopy phenology during the dry season using a tower hyperspectral camera and modis observations. *ISPRS Journal of Photogrammetry and Remote Sensing* **131**: 52-64.

Díaz S, Kattge J, Cornelissen JH, Wright IJ, Lavorel S, Dray S, Reu B, Kleyer M, Wirth C, Prentice IC *et al.* 2016. The global spectrum of plant form and function. *Nature* **529:** 167-171.

Domingues TF, Meir P, Feldpausch TR, Saiz G, Veenendaal EM, Schrodt F, Bird M, Hien F, Compaore H, Aiallo A *et al.* 2010. Co-limitation of photosynthetic capacity by nitrogen and phosphorus in West Africa woodlands. *Plant, Cell & Environment* **33:** 959-980.

Domingues TF, Martinelli LA, Ehleringer JR. 2014. Seasonal patterns of leaf-level photosynthetic gas exchange in an eastern Amazonian rain forest. *Plant Ecology & Diversity* **7:** 189-203.

Dong N, Prentice IC, Evans BJ, Caddy-Retalic S, Lowe AJ, Wright IJ. 2017. Leaf nitrogen from first principles: field evidence for adaptive variation with climate. *Biogeosciences* **14:** 481–495.

Doughty CE, Asner GP, Martin RE. 2011. Predicting tropical plant physiology from leaf and canopy spectroscopy. *Oecologia* **165:** 289-299.

Elvidge CD. 1990. Visible and near infrared reflectance characteristics of dry plant materials. *International Journal of Remote Sensing* **11:** 1775-1795.

Evans JR, Clark VC. 2019. The nitrogen cost of photosynthesis. *Journal of Experimental Botany* **70:** 7-15.

Farquhar GV, von Caemmerer SV, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. *Planta* **149:** 78-90.

Field C. 1983. Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. *Oecologia* **56:** 341-347.

Geladi P, Kowalski BR. 1986. Partial least-squares regression: a tutorial. *Analytica Chimica Acta* **185:** 1-17.

Guanter L, Kaufmann H, Segl K, Foerster S, Rogass C, Chabrillat S, Kuester T, Hollstein A, Rossner G, Chlebek C, *et al.* 2015. The EnMAP spaceborne imaging spectroscopy mission for earth observation. *Remote Sensing* **7:** 8830-8857.

Han Q, Kawasaki T, Nakano T, Chiba Y. 2008. Leaf-age effects on seasonal variability in photosynthetic parameters and its relationships with leaf mass per area and leaf nitrogen concentration within a Pinus densiflora crown. *Tree Physiology* **28:** 551-558.

Hutyra LR, Munger JW, Saleska SR, Gottlieb E, Daube BC, Dunn AL, Amaral DF, de Camargo PB, Wofsy SC. 2007. Seasonal controls on the exchange of carbon and water in an Amazonian rain forest. *Journal of Geophysical Research: Biogeosciences* **112:** G03008, https://doi.org/10.1029/2006JG000365.

Jacob J, Greitner C, Drake BG. 1995. Acclimation of photosynthesis in relation to Rubisco and non-structural carbohydrate contents and in situ carboxylase activity in Scirpus olneyi grown at elevated CO_2 in the field. *Plant, Cell & Environment* **18:** 875-884.

Kattge J, Knorr W. 2007. Temperature acclimation in a biochemical model of photosynthesis: a reanalysis of data from 36 species. *Plant, Cell & Environment* **30:** 1176-1190.

Kattge J, Diaz S, Lavorel S, Prentice IC, Leadley P, Bönisch G, Garnier E, Westoby M, Reich PB, Wright IJ *et al.* 2011. TRY–a global database of plant traits. *Global Change Biology* **17:** 2905-2935.

Kenzo T, Ichie T, Watanabe Y, Yoneda R, Ninomiya I, Koike T. 2006. Changes in photosynthesis and leaf characteristics with tree height in five dipterocarp species in a tropical rain forest. *Tree Physiology* **26:** 865-873.

686 **Kim Y, Knox RG, Longo M, Medvigy D, Hutyra LR, Pyle EH, Wofsy SC, Bras RL,**
687 **Moorcroft, PR. 2012.** Seasonal carbon dynamics and water fluxes in an Amazon
688 rainforest. *Global Change Biology* **18:** 1322-1334.
689 **Kitajima K, Mulkey SS, Samaniego M, Wright J.S. 2002.** Decline of photosynthetic capacity
690 with leaf age and position in two tropical pioneer tree species. *American Journal of*
691 *Botany* **89:** 1925-1932.
692 **Kokaly RF, Asner GP, Ollinger SV, Martin ME, Wessman CA. 2009.** Characterizing canopy
693 biochemistry from imaging spectroscopy and its application to ecosystem studies. *Remote*
694 *Sensing of Environment* **113:** S78-S91.
695 **Kumarathunge DP, Medlyn BE, Drake JE, Tjoelker MG, Aspinwall MJ, Battaglia M,**
696 **Cano FJ, Carter KR, Cavaleri MA, Cernusak LA, Chamber JQ, Crous KY *et al.***
697 **2019.** Acclimation and adaptation components of the temperature dependence of plant
698 photosynthesis at the global scale. *New Phytologist* **222:** 768-784.
699 **Long SP, Bernacchi CJ. 2003.** Gas exchange measurements, what can they tell us about the
700 underlying limitations to photosynthesis? Procedures and sources of error. *Journal of*
701 *Experimental Botany* **54**: 2393-2401.
702 **Lopes AP, Nelson BW, Wu J, de Alencastro GPML, Tavares JV, Prohaska N, Martins GA,**
703 **Saleska SR. 2016.** Leaf flush drives dry season green-up of the central Amazon. *Remote*
704 *Sensing of Environment* **182:** 90-98.
705 **Medlyn BE, Badeck FW, De Pury DGG, Barton CVM, Broadmeadow M, Ceulemans R, De**
706 **Angelis P, Forstreuter M, Jach ME, Kellomäki S *et al.* 1999.** Effects of elevated [CO_2]
707 on photosynthesis in European forest species: a meta-analysis of model
708 parameters. *Plant, Cell & Environment* **22:** 1475-1495.
709 **Muraoka H, Saigusa N, Nasahara KN, Noda H, Yoshino J, Saitoh TM, Nagai S, Murayama**
710 **S, Koizumi H. 2010.** Effects of seasonal and interannual variations in leaf photosynthesis
711 and canopy leaf area index on gross primary production of a cool-temperate deciduous
712 broadleaf forest in Takayama, Japan. *Journal of Plant Research* **123:** 563-576.
713 **National Academies of Sciences E, Medicine. 2018.** *Thriving on Our Changing Planet: A*
714 *Decadal Strategy for Earth Observation from Space*. Washington, DC: The National
715 Academies Press.

716 **Nepstad DC, Moutinho P, Dias-Filho MB, Davidson E, Cardinot G, Markewitz D,**
717 **Figueiredo R, Vianna N, Chambers J, Ray D *et al.* 2002.** The effects of partial
718 throughfall exclusion on canopy processes, aboveground production, and
719 biogeochemistry of an Amazon forest. *Journal of Geophysical Research:*
720 *Atmospheres* **107**: https://doi.org/10.1029/2001JD000360.
721 **Niinemets, U. 2016.** Leaf age dependent changes in within-canopy variation in leaf functional
722 traits: a meta-analysis. *Journal of Plant Research* **129:** 313–338.
723 **Norby RJ, Gu L, Haworth IC, Jensen AM, Turner BL, Walker AP, Warren JM, Weston**
724 **DJ, Xu C, Winter K. 2017.** Informing models through empirical relationships between
725 foliar phosphorus, nitrogen and photosynthesis across diverse woody species in tropical
726 forests of Panama. *New Phytologist* **215:** 1425-1437.
727 **Ollinger SV. 2011.** Sources of variability in canopy reflectance and the convergent properties of
728 plants. *New Phytologist* **189:** 375-394.
729 **Onoda Y, Hikosaka K, Hirose T. 2004.** Seasonal change in the balance between capacities of
730 RuBP carboxylation and RuBP regeneration affects CO_2 response of photosynthesis in
731 Polygonum cuspidatum. *Journal of Experimental Botany* **56:** 755-763.
732 **Pantin F, Simonneau T, Muller B. 2012.** Coming of leaf age: control of growth by hydraulics
733 and metabolics during leaf ontogeny. *New Phytologist* **196:** 349-366.
734 **Reich PB, Uhl C, Walters MB, Prugh L, Ellsworth DS. 2004.** Leaf demography and
735 phenology in Amazonian rain forest: a census of 40000 leaves of 23 tree
736 species. *Ecological Monographs* **74:** 3-23.
737 **Ricciuto D, Sargsyan K, Thornton P. 2018.** The impact of parametric uncertainties on
738 biogeochemistry in the E3SM land model. *Journal of Advances in Modeling Earth*
739 *Systems* **10:** 297-319.
740 **Rice AH, Pyle EH, Saleska SR, Hutyra L, Palace M, Keller M, De Camargo PB, Portilho K,**
741 **Marques DF, Wofsy SC. 2004.** Carbon balance and vegetation dynamics in an
742 old-growth Amazonian forest. *Ecological Applications* **14:** 55-71.
743 **Roberts DA, Nelson BW, Adams JB, Palmer F. 1998.** Spectral changes with leaf aging in
744 Amazon caatinga. *Trees* **12**: 315-325.
745 **Rogers A. 2014.** The use and misuse of Vc,max in Earth System Models. *Photosynthesis*
746 *Research* **119:** 15-29.

Rogers A, Medlyn BE, Dukes JS, Bonan G, Caemmerer S, Dietze MC, Kattge J, Leakey AD, Mercado LM, Niinemets Ü *et al*. 2017a. A roadmap for improving the representation of photosynthesis in Earth system models. *New Phytologist* **213:** 22-42.

Rogers A, Serbin SP, Ely KS, Sloan VL, Wullschleger SD. 2017b. Terrestrial biosphere models underestimate photosynthetic capacity and CO_2 assimilation in the Arctic. *New Phytologist* **216:** 1090-1103.

Rosipal R, Krämer N. 2006. Overview and recent advances in partial least squares. In Saunders, C., Grobelink, M., Gunn, S., & Shawe-Taylor, J. (Eds.), *Subspace, Latent Structure and Feature Selection*. Berlin: Springer, 34-51.

Scafaro AP, Xiang S, Long BM, Bahar NH, Weerasinghe LK, Creek D, Evans JR, Reich PB, Atkin OK. 2017. Strong thermal acclimation of photosynthesis in tropical and temperate wet-forest tree species: the importance of altered Rubisco content. *Global Change Biology* **23**: 2783-2800.

Schimel D, Pavlick R, Fisher JB, Asner GP, Saatchi S, Townsend P, Miller C, Frankenberg C, Hibbard K, Cox P. 2015. Observing terrestrial ecosystems and the carbon cycle from space. *Global Change Biology* **21:** 1762-1776.

Schneider FD, Morsdorf F, Schmid B, Petchey OL, Hueni A, Schimel DS, Schaepman ME. 2017. Mapping functional diversity from remotely sensed morphological and physiological forest traits. *Nature Communications* **8:** 1441-1441, https://doi.org/10.1038/s41467-017-01530-3.

Schweiger AK, Cavender-Bares J, Townsend PA, Hobbie SE, Madritch MD, Wang R, Tilman D, Gamon J A. 2018. Plant spectral diversity integrates functional and phylogenetic components of biodiversity and predicts ecosystem function. *Nature Ecology & Evolution* **2:** 976-982.

Serbin SP, Dillaway DN, Kruger EL, Townsend PA. 2012. Leaf optical properties reflect variation in photosynthetic metabolism and its sensitivity to temperature. *Journal of Experimental Botany* **63:** 489-502.

Serbin SP, Singh A, McNeil BE, Kingdon CC, Townsend PA. 2014. Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. *Ecological Applications* **24:** 1651-1669.

Serbin SP, Singh A, Desai AR, Dubois SG, Jablonski AD, Kingdon CC, Kruger EL, Townsend PA. 2015. Remotely estimating photosynthetic capacity, and its response to temperature, in vegetation canopies using imaging spectroscopy. *Remote Sensing of Environment* **167:** 78-87.

Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL. 2007. Fitting photosynthetic carbon dioxide response curves for C3 leaves. *Plant, Cell & Environment* **30:** 1035-1040.

Silva-Perez V, Molero G, Serbin SP, Condon AG, Reynolds MP, Furbank RT, Evans JR. 2018. Hyperspectral reflectance as a tool to measure biochemical and physiological traits in wheat. *Journal of Experimental Botany* **3:** 483-496.

Singh A, Serbin SP, McNeil BE, Kingdon CC, Townsend PA. 2015. Imaging spectroscopy algorithms for mapping canopy foliar chemical and morphological traits and their uncertainties. *Ecological Applications* **25:** 2180-2197.

Smith NJ, Keenan TF, Prentice IC, Wang H, Wright IJ, Niinemets U, Crous KY, Domingues TF, Guerrieri R, Ishida FY *et al*. 2019. Global photosynthetic capacity is optimized to the environment. *Ecology Letters* **22:** 506-517.

Sobrado MA. 1994. Leaf age effects on photosynthetic rate, transpiration rate and nitrogen content in a tropical dry forest. *Physiologia Plantarum* **90:** 210-215.

Sonnleitner M, Dullinger S, Wanek W, Zechmeister H. 2009. Microclimatic patterns correlate with the distribution of epiphyllous bryophytes in a tropical lowland rain forest in Costa Rica. *Journal of Tropical Ecology* **25:** 321-330.

Sperry J. 2013. Cutting-edge research or cutting-edge artefact? An overdue control experiment complicates the xylem refilling story. *Plant, Cell & Environment* **36**: 1916-1918.

Stavros EN, Schimel D, Pavlick R, Serbin S, Swann A, Duncanson L, Fisher JB, Fassnacht F, Ustin S, Dubayah R *et al*. 2017. ISS observations offer insights into plant function. *Nature Ecology and Evolution* **1:** 0194.

Steege H, Pitman NC, Sabatier D, Baraloto C, Salomão RP, Guevara JE, Phillips OL, Castilho CV, Magnusson WE, Molino JF *et al*. 2013. Hyperdominance in the Amazonian tree flora. *Science* **342:** 1243092.

Stinziano JR, Morgan PB, Lynch DJ, Saathoff AJ, McDermitt DK, Hanson DT. 2017. The rapid A–Ci response: photosynthesis in the phenomic era. *Plant, Cell & Environment* **40:** 1256-1262.

Turner BL, Romero TE. 2009. Short-term changes in extractable inorganic nutrients during storage of tropical rain forest soils. *Soil Science Society of America Journal* **73:** 1972-1979.

Walker AP, Beckerman AP, Gu L, Kattge J, Cernusak LA, Domingues TF, Scales JC, Wohlfahrt G, Wullschleger SD, Woodward FI. 2014. The relationship of leaf photosynthetic traits– V_{cmax} and J_{max}–to leaf nitrogen, leaf phosphorus, and specific leaf area: a meta-analysis and modeling study. *Ecology and Evolution* **4:** 3218-3235.

Walker AP, Quaife T, Bodegom PM, De Kauwe MG, Keenan TF, Joiner J, Lomas MR, MacBean N, Xu C, Yang X *et al.* 2017. The impact of alternative trait-scaling hypotheses for the maximum photosynthetic carboxylation rate (V_{cmax}) on global gross primary production. *New Phytologist* **215:** 1370-1386.

Wilson KB, Baldocchi DD, Hanson PJ. 2001. Leaf age affects the seasonal pattern of photosynthetic capacity and net ecosystem exchange of carbon in a deciduous forest. *Plant, Cell & Environment* **24:** 571-583.

Wolter PT, Townsend PA, Sturtevant BR, Kingdon CC. 2008. Remote sensing of the distribution and abundance of host species for spruce budworm in Northern Minnesota and Ontario. *Remote Sensing of Environment* **112:** 3971-3982.

Wright SJ, Horlyck V, Basset Y, Barrios H, Bethancourt A, Bohlman SA, Gilbert GS, Goldstein G, Graham EA, Kitajima K *et al.* 2003. Tropical canopy biology program, Republic of Panama. *Studying forest canopies from above: the International Canopy Crane Network,* eds Basset, Y., Horlyck, V., & Wright, S.J. (Smithsonian Tropical Research Institute and the United Nations Environmental Programme, Panama City, Panama), 137-155.

Wu J, Albert LP, Lopes AP, Restrepo-Coupe N, Hayek M, Wiedemann KT, Guan K, Stark SC, Christoffersen B, Prohaska N *et al.* 2016. Leaf development and demography explain photosynthetic seasonality in Amazon evergreen forests. *Science* **351:** 972-976.

Wu J, Chavana-Bryant C, Prohaska N, Serbin SP, Guan K, Albert LP, Yang X, Leeuwen WJ, Garnello AJ, Martins G *et al.* 2017. Convergence in relationships between leaf traits, spectra and age across diverse canopy environments and two contrasting tropical forests. *New Phytologist* **214:** 1033-1048.

Wu J, Kobayashi H, Stark SC, Meng R, Guan K, Tran NN, Gao S, Yang W, Restrepo-Coupe N, Miura T *et al*. 2018. Biological processes dominate seasonality of remotely sensed canopy greenness in an Amazon evergreen forest. *New Phytologist* **217:** 1507-1520.

Xu QS, Liang YZ. 2001. Monte Carlo cross validation. *Chemometrics and Intelligent Laboratory Systems* **56:** 1-11.

Xu X, Medvigy D, Wright JS, Kitajima K, Wu J, Albert LP, Martins GA, Saleska SR, Pacala SW. 2017. Variations of leaf longevity in tropical moist forests predicted by a trait-driven carbon optimality model. *Ecology Letters* **20:** 1097-1106.

Yendrek C, Tomaz T, Montes CM, Cao Y, Morse AM, Brown PJ, McIntyre L, Leakey A, Ainsworth E. 2017. High-throughput phenotyping of maize leaf physiology and biochemistry using hyperspectral reflectance. *Plant Physiology* **173:** 614-626.

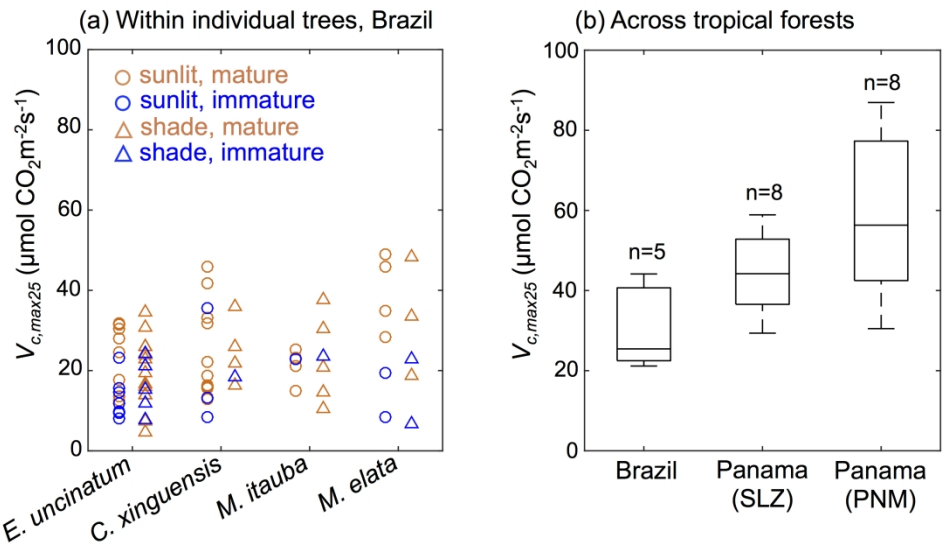
851 **Figure legends**

852 **Figure 1.** Large variation in leaf $V_{c,max25}$ (a) within individual trees and (b) across tropical
853 forests. The data were from the Tapajos K67 site in Brazil, the San Lorenzo crane site (SLZ) and
854 the Parque Natural Metropolitano crane site (PNM) in The Republic of Panama. At the
855 individual tree level (panel a), $V_{c,max25}$ is primarily associated with leaf development (orange for
856 variation in mature leaves, and blue for variation in immature leaves; see Methods) and canopy
857 position (circles for the leaves sampled from the sunlit canopy environment, and triangles for the
858 leaves sampled from the shaded canopy environment). Across tropical forests (panel b), the
859 spread of tree specific (sunlit canopy, mature leaves) mean $V_{c,max25}$ of each forest site is displayed
860 with a boxplot, in which the central mark is the median, the edges are the 25th and 75th
861 percentiles, and the whiskers are the 5th and 95th percentiles.

862 **Figure 2.** A spectra-$V_{c,max25}$ model was trained using two thirds of the dataset from mature leaves
863 measured in Panama, and then evaluated using the independent validation dataset collected in (a)
864 Panamanian mature leaves, (b) Panamanian immature leaves, (c) Brazilian mature and immature
865 leaves, and (d) all leaf classes collected in Panama and Brazil. Error bars denote the 95%
866 confidence intervals for each predicted value based on the ensemble PLSR models (i.e. each
867 PLSR model is represented by a set of PLSR fitted spectral coefficients, and in total includes 100
868 Monte-Carol model runs; see Methods); the gray line shows the ordinary least square regression
869 fit, and the black line shows the 1:1 line.

870 **Figure 3.** The final spectra-$V_{c,max25}$ model was trained using two thirds of our entire dataset, and
871 then applied to the remaining, independent validation dataset. Error bars denote the 95%
872 confidence intervals for each predicted value based on the ensemble PLSR models, the gray line
873 shows the ordinary least square regression fit, and the black line shows the 1:1 line.

874 **Figure 4.** The combination of our final spectral model for $V_{c,max25}$ (Fig. 3) and the leaf age model
875 (see Wu *et al.,* 2017; also see Methods) enables the prediction of the life history trajectories of
876 leaf $V_{c,max25}$ (grey circles) in an independent spectra-age dataset collected in Brazil (see
877 Methods). Here we show that for a given leaf age (determined by the spectra model) we can
878 capture the dynamics of field observed $V_{c,max25}$ (red circles). See Table S1 for the full species
879 names, error bars denote the 95% confidence interval of spectral predictions.



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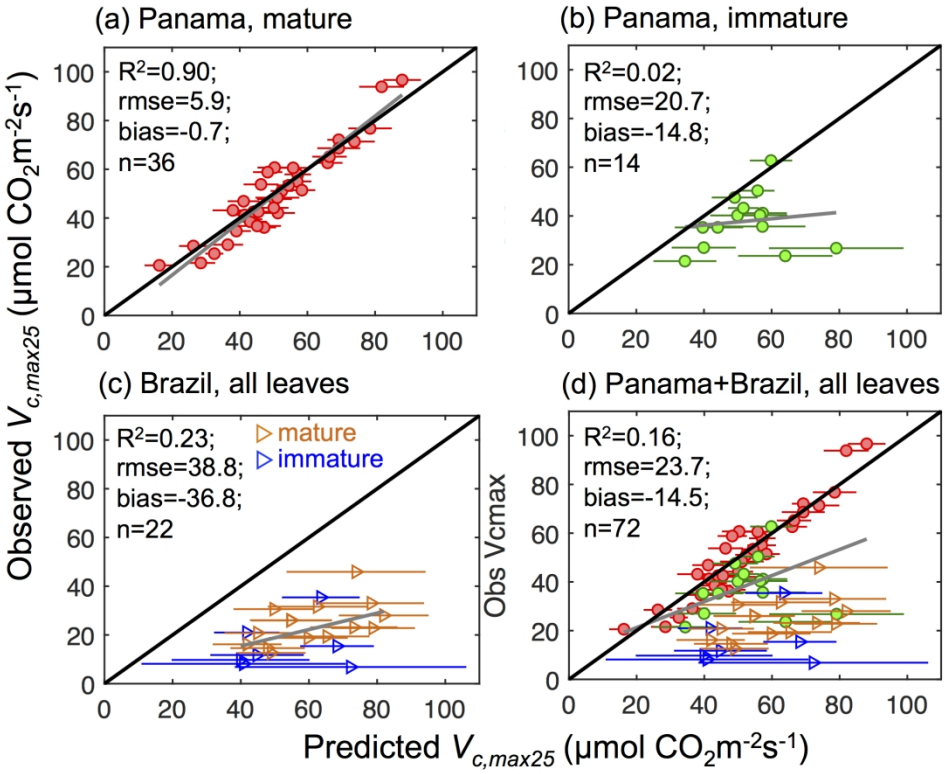

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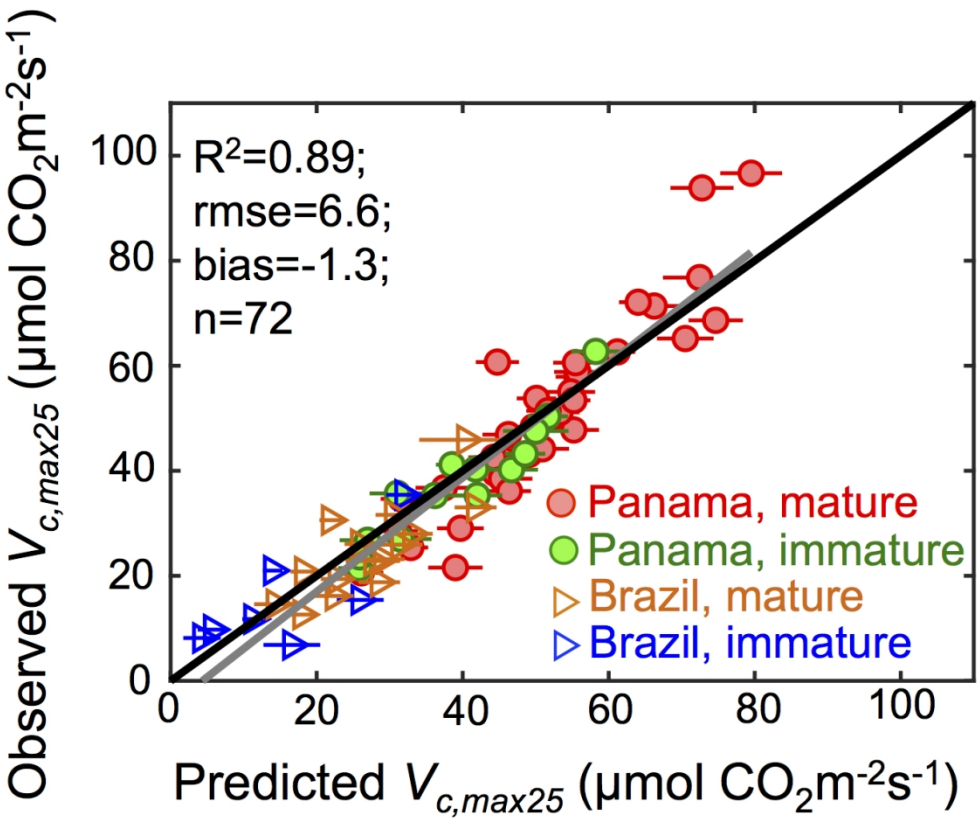

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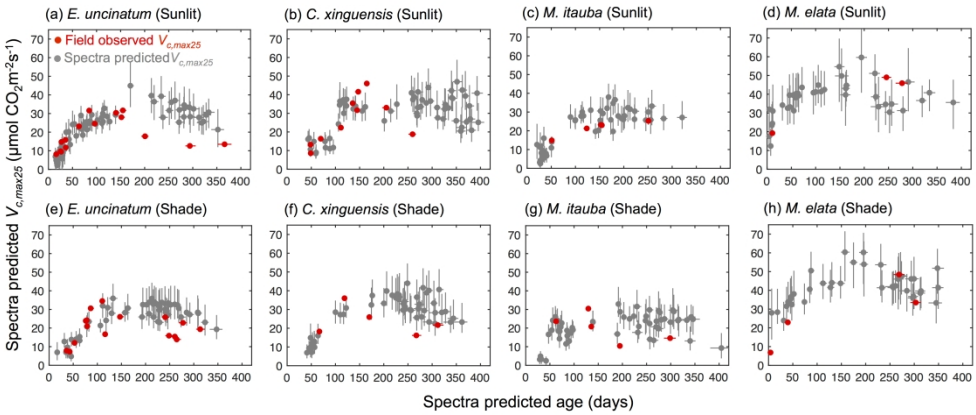

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